Comparison Of Efficacy Of Cell Block Versus Conventional Smear Cytology Study In Serous Effusions

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Abstract: Background: Diagnostic cytology is study of cells that are exfoliated from epithelial surface or remove from the tissue. Cytological examination of body fluid helps us to differentiate inflammatory effusion from malignant effusion. It has diagnostic, prognostic and therapeutic implications. The method has disadvantage of lower sensitivity to detected and differentiate malignant cell from reactive atypical mesothelial cells. Aims: The aim of this study is to compare the cytological features of conventional smear (CS) method and cell block (CB) method in serous effusions and access their ability to diagnose malignancy. Material And Methods: Nighty seven serous effusion (pleural and peritoneal) samples were subjected to evaluation by both CS and CB methods.. Each fluid specimen was divided into two equal parts, Cs and CB prepared. Cell blocks were prepared using plasma-thromboplastin cell block technique. Cellularity, architecture patterns, morphological features and yield for malignancy were compared, using the two methods. Result: Among 97 cases common age was 41-60 year with M:F sex ratio 1.06:1. Most common cause of effusion were Pulmonary tuberculosis (20.69%) followed by Ovarian mass (19.59%), Liver cirrhosis (6.19%), Lung mass (5.15%).Cell block was diagnostically superior (Score 6-8) in 34.02% cases whereas no cases were found diagnostically superior with conventional smear. The additional yield of malignancy was found to be 11.94% more by cell block method compared to conventional smear. Conclusion: The CB method provides high cellularity, better architectural patterns, morphological features and an additional yield of malignant cells, and thereby, increases the sensitivity of the cytodiagnosis when compared with the CS method. [Dehariya C Natl J Integr Res Med, 2020; 11(4):10-14]

Key Words: Cell block, conventional smear, serous effusions, Malignancy.

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Introduction: Diagnostic cytology is study of cells that are exfoliated from epithelial surface or remove from the tissue. It has diagnostic, prognostic and therapeutic implications[1]. Cytological examination of body fluid help us to differentiate inflammatory effusion from malignant effusion. Accurate identification of malignant or reactive mesothelial cell is a diagnostic problem in conventional cytology due to overcrowding of cells, loss of tissue architecture and cell loss due to useful material left behind in centrifuge tube. The cell block technique of examining the fluids, along with concomitant use of smears has shown an added advantage in such cases. The main advantages of cellblock technique are preservation of tissue architecture, increase cell yield and also obtain multiple sections from the same material for special stains, so that cell block increase sensitivity and specificity[2].

Cell block preparation with conventional techniques such as agar gel or formol-alcohol is laborious and time-consuming. Therefore, in the present study, plasma-thromboplastin cell block technique (CB) was performed. This technique is simple, cost-effective and readily adaptable in routine hospital laboratories[3].

The purpose of this study was to access and compare findings of conventional smear and cell block method in different body cavity effusions.

Material & Methods: This observational comparative study was conducted in department of pathology of a tertiary care hospital over a period of one and half year from January 2018 to June 2019 in central India, after ethical approval by institutional ethical and research committee. A total of 97 body fluid samples were obtained from patients of various departments. Fresh body fluids minimum of Ten ml in two separate containers (from plural and peritoneal cavity) were included in study while scanty fluid samples were excluded. The clinical information including age, sex, history, provisional diagnosis was noted. Ten millilitres of fresh serous pleural and peritoneal samples were received first submitted for physical examination then samples divided into two containers (5ml each) for conventional cytology smear and cell block technique.

- First container was taken for cytology transfer in centrifugation tube and centrifuged at 2000 RPM for 15 minutes. Three thin smears prepared from the

sediment. Two slides were fixed in 95% methanol and stained with haematoxylin and eosin stain, other one dry smear was made and stained with May-Grunwald Giemsa (MGG) stain.

- Second container was taken for cell block transfer in centrifugation tube and centrifuged at 2000 RPM for 15 minutes. Supernatant was discarded, and 2-3 drops of pooled plasma and 2-3 drops of thromboplastin reagent were added to the sediment and centrifuged again for 3 minutes for proper aggregating and condensation of sediment. The cell button sediment was put on filter paper, wrapped and kept in 10% formalin for 8 hour for fixation. Thereafter cell button was processed in histokinette as a part of routine paraffin embedded section preparation. The paraffin sections were stained with haematoxylin and eosin stain and mounted with DPX moutent.

Quality of smears and cell blocks was assessed according to Miar's criteria, as follows[2,4:]

- Volume of blood/clot obscuring background (large: 0, moderate: 1, minimal: 2)
- Amount of diagnostic cellular material present.(minimal: 0, moderate: 1, abundant: 2)
- Degree of cellular degeneration and cellular trauma.(marked: 0, moderate: 1, minimal: 2)
- Retained architecture/cellular arrangement. (minimal: 0, moderate: 1, excellent: 2).

Zero to two point score was given to individual smear/ cell blocks based on each of the above criteria, and the final score was calculated by adding the scores of four criterions. Qualitative grouping of smears and cell blocks was done into three categories:[5]

- Diagnostically unsuitable – (0-2 score)
- Diagnostically adequate – (3–5 score)
- Diagnostically superior – (6–8 score)

The cytological smears and block sections were examined separately for cellularity, architectural patterns and morphology (cytoplasmic and nuclear details) to render a cytological diagnosis for each case, and the findings were compared. Cytomorphological features were studied to identify the malignancy and the most probable primary site. Yield for malignancy was identified by both the methods. Diagnostic accuracy of both conventional smear and cell block method for diagnosing malignancy was calculated.

Results: The present study was conducted on 97 patients who underwent paracentesis for the diagnosis of effusion cytology by conventional smear and cell block techniques. The Methods were also assessed for the utility and sensitivity of conventional smear and cell block technique in body fluid cytodiagnosis.

Among 97 cases, most common age group was age 41-60 year which included 61.8 % of the patients with slight difference in sex (50 (51.55%) were male and 47(49.45%) were female). As far as source of body fluid is concern, 52 (53.60%) samples were ascetic fluids and 45 (46.39%) samples were pleural fluids.

Based on clinical diagnosis (Figure-1) most common causes of effusion were Pulmonary tuberculosis (20.69%) followed by Ovarian mass (19.59%), Liver cirrhosis (6.19%), Lung mass (5.15%), while carcinoma stomach (1.03%), carcinoma colon(1.03%) , pott's spine(1.03%) etc were least common of the clinical diagnosis.

Figure 1: Distribution Of Cases According To Clinical Diagnosis.

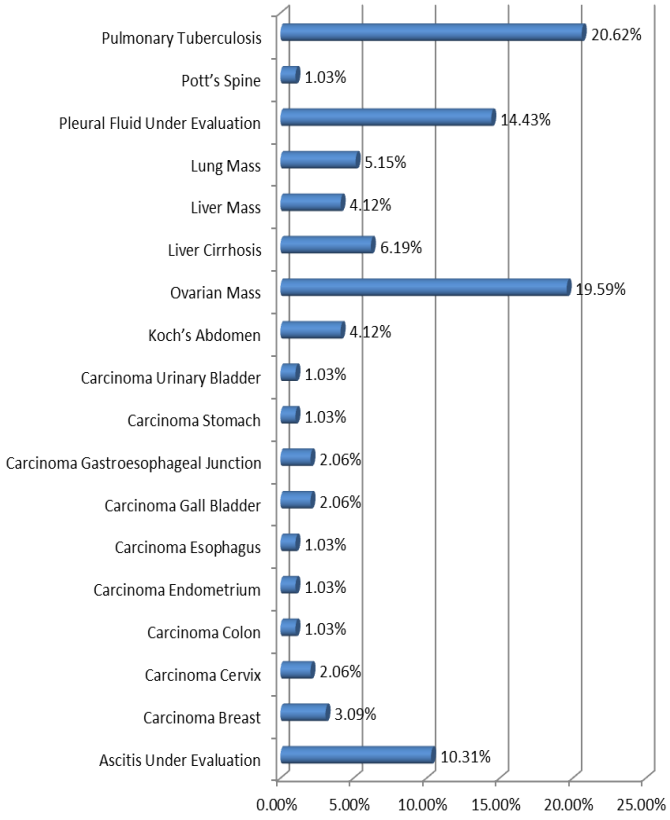

Table 1: Comparative Evaluation Of Conventional Smear And Cell Block Cytology

Parameters	Conventional smear		Cell Block		Chi- Square (p-value)
	Number of Patients	Per-cent	Number of Patients	Per-cent	
Background Blood					
Score 0	35	36.08 %	18	18.56 %	<0.0001
Score 1	59	60.82 %	41	42.27 %	
Score 2	03	3.09 %	38	39.18 %	
Amount Of Diagnostic Material (Cellularity)					
Score 0	48	49.48 %	41	42.27 %	0.0009
Score 1	49	50.52 %	43	44.33 %	
Score 2	00	0 %	13	13.4 %	
Degree Of Cellular Degeneration					
Score 0	35	36.08 %	10	10.31 %	<0.0001
Score 1	59	60.82 %	35	36.08 %	
Score 2	03	3.09 %	52	53.61 %	
Distribution Of Cells					
Score 0	62	63.92 %	32	32.99 %	<0.0001
Score 1	35	36.08 %	41	42.27 %	
Score 2	00	0 %	24	24.74 %	

Table 2: Descriptive Statistic For Each Of The Four Parameters Of Conventional Smear And Cell Block Cytology

Parameters	Conventional Smear (Mean±SD)	Cell Block (Mean±SD)	T-Test
Background	0.67 ± 0.53	1.21 ± 0.74	<0.0001
Cellularity	0.51 ± 0.5	0.71 ± 0.69	0.0004
Disintegration	0.67 ± 0.53	1.43 ± 0.68	<0.0001
Distribution	0.36 ± 0.48	0.92 ± 0.76	<0.0001

As shown in Table 1 and 2, cell block cytology was found to be significantly better than conventional smear ($p<0.05$). The yield of cells with details of architectural pattern information about cellular and nuclear features, were more accurate in cell block as compared to the conventional smear method.

It has been observed that conventional smear show individually dispersed cells, clusters, papillary fragments and acinar formations, signet ring cells or keratinised cells in malignant fluid effusions, but the appreciation of architectural pattern of the malignant cytology, such as, three dimensional clusters, cell balls, sheets, cellular as well as nuclear pleomorphism, nuclear hyperchromatism, irregularity of the nuclear contours, type of chromatin, prominence of nucleoli, atypical mitotic figures and features of differentiation such as intracellular secretions, signet ring cells and evidence of keratinisation is much better perceived in cell blocks (Figure 2).

In present study, it can be observed that 34.02% cases have cell block diagnostically superior whereas no cases were found to be diagnostically superior with conventional smear (Table-3). While only 50 (52.55 %) smears were diagnostically adequate to make diagnosis with conventional smear method while 67 (69.07 %) cases with cell block.

Figure 2: Photomicrograph Of Cell Block Showing Architectural Arrangement, Acinar Pattern, Signet Ring Cell And Other Features Of Malignancy.

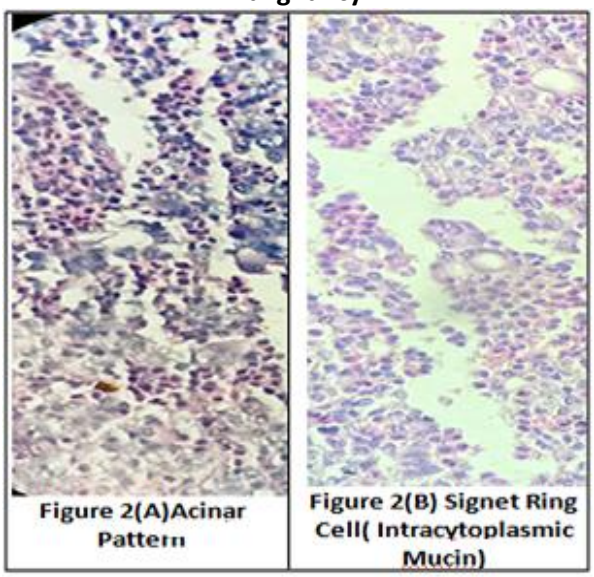

Figure 2(A)Acinar Pattern

Figure 2(B) Signet Ring Cell(Intracytoplasmic Mucin)

Table 3: Diagnostic Scores Of Conventional And Cell Block Sections

Diagnostic Score	Conventional Smear		Cell Block		Chi- Square (P-Value)
	Number Of Patients	Percent	Number Of Patients	Percent	
Diagnostically Superior (6-9)	0	0 %	33	34.02 %	0.0310
Diagnostically Adequate (3-5)	50	51.55 %	34	35.05 %	
Diagnostically Inadequate (0-2)	47	48.45 %	30	30.93 %	
Total	97	100%	97	100%	

After the analysis of fluid samples, including ascitic and pleural fluids, results were categorised as benign (Both inflammatory and negative for malignancy), suspicious for malignancy and as malignant effusions. Chi square analysis was used to compare proportion of the benign and malignant lesions diagnosed by conventional smear & cell block method which revealed significant diagnostic capability of cell block method compared to Cell smear (Table-4).

Table 4: Comparison Of Cytological Diagnosis On Conventional Smear And Cell Block Study

Diagnosis	Conventional Smear		Cell Block		Chi- Square (P-Value)
	Number Of Patients	Percent	Number Of Patients	Percent	
Benign	41	82 %	45	67.16 %	<0.0001
Suspicious	9	18 %	14	20.9 %	
Malignant	0	0 %	8	11.94 %	
Total	50	100%	67	100%	

Discussion: The cytological examination of the serous effusions is a routinely done procedure in cytology laboratories of pathology everywhere. It is of considerable importance in various benign conditions like hepatic cirrhosis, pleurisy, pulmonary infarcts and in suspected malignant effusions as well as in staging in patients with known primary malignancy. Examination of fluid cytology is of paramount importance and has the diagnostic, therapeutic and prognostic implications. The presence of malignant cells in the pleural or ascitic fluid are almost always indicative of metastasis and advanced stage of malignancy as the primary malignancies of the mesothelial lining are very rare. This investigation helps to detect unsuspected cancers and the metastasis from known primary.

Among 97 cases common age was 41-60 year with slight difference in sex. M : F sex ratio was 1.06:1. These finding were comparable with Bansode et al[6] and Padmavathi et al[7] who have reported modal number of cases in the age group 41–60 years as 54% and 69.3%, respectively, while Matreja et al[8] found peek age 21-30 year. Similar to our study Matreja et al[8] and padmavathi et al[7] also found M : F ratio of 1: 0.98 and 1.4 : 1 respectively. As source of body fluid was ascitic in 53.60% samples and pleural in 46.39% samples. Similar to present study Poorana et al[9] also reported 48.33% samples of peritoneal fluid and 45.83% of pleural fluid while 3.33% were synovial and 2.5% were pericardial. Matreja et al[8] on other hand found 41.8% ascetic and 58.2% plural fluid. It might be due to more prevalent pulmonary diseases in that area.

Most common cause of effusion was Pulmonary tuberculosis (20.69%) followed by Ovarian mass (19.59%), Liver cirrhosis (6.19%), Lung mass (5.15%) while carcinoma stomach (1.03%), carcinoma colon(1.03%), pott's spine (1.03%) etc least common.(table -1). Similar proportion of causes also found by Thapar et al[2] in their study where pulmonary tuberculosis and liver cirrhosis were common causes of effusion.

When adequacy for diagnosis was assessed for both techniques, In present study, cell block was diagnostically superior (Score 6-8) in 34.02% cases whereas no cases were found diagnostically superior with conventional smear. Diagnostically adequate sample were also more with cell block method (Table-3). Similarly Bista et al[5] observed that cell block have 67.51% diagnostically superior smears i.e. (score 6-8) as compared to 43% of conventional smears. On the other hand Bista et al reported more

adequate smears for diagnosis (Score 3-5) in conventional smears cytology techniques (35.13%) than cell blocks (8.1 %).

The use of cell block not only increases the cellularity as compared to routine centrifuge, but also the cells were evenly distributed. Not only cellular morphology, nuclear and cytoplasmic details were better on cell block technique but also cell block carries additional advantage of performing immunohistochemistry which aids in the diagnosis and can be used for retrospective analysis[2].

Cytological diagnosis of effusion fluid samples, that were diagnostically adequate (50 samples with conventional smear while 67 samples with cell block) including ascetic and pleural fluids, results were categorised as benign (Both inflammatory and negative for malignancy), suspicious for malignancy or malignant effusions. Cell Block method is significantly ($p<0.0001$) better in in the detection of malignancy which detected 11.94% samples as malignant in the same cohort of sample conventional method detected none (Table-4).

In a study by Matreja et al [8] diagnostic yield of malignancy was 6.53% on conventional smear examination which was increased to 8.5% by cell block method. Similarly in a study by Bansode et.al[6], 15% yield for malignancy on conventional smear was increased to 18% on cell block study. These results were similar to ours for cell block method. The additional yield of malignancy was found to be 11.94% more by cell block method compared to that attained by conventional smear in the present study.

Conclusion: We conclude that the cell block technique is simple, inexpensive and does not require any special training or instrument. The cell block method provides high cellularity, better architectural patterns, better morphological features, additional yield of malignant cells and increased sensitivity for cytodiagnosis of malignant lesions as compared to the conventional smear method.

The cells block help particularly in cases where there is a diagnostic dilemma between the malignancy and reactive changes. Cell block preparations can be combined with conventional smear wherever possible to improve diagnostic accuracy and reach definitive diagnosis.

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